# Introduction to the gonadal staging standards of Chinese scientific observers for billfish and estimation of size-at-maturity

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**Abstract**

$L_{50}$ (size at 50% maturity) is an important concept in fish reproductive biology and a critical parameter in integrated stock assessment models. The estimation of $L_{50}$ largely depends on the data sources and biological criteria used to determine maturity. This paper briefly introduces the gonadal staging standards for tuna and tuna-like species used in the China Scientific Observer Program, and estimated $L_{50}$ for four billfish species based on functional and physiological maturity data in the Western Indian Ocean, respectively. Our results indicate that the estimated $L_{50}$ based on functional maturity data for indo-pacific sailfish, black marlin, striped marlin, and blue marlin were 187.04 cm, 174.59 cm, 170.05 cm, and 179.95 cm, respectively. After adjusting 8, 1, 6, and 9 individuals with stage-III from immature to mature due to their larger body size, the estimated $L_{50}$ based on physiological maturity data for the four billfish species were 177.55 cm, 172.67 cm, 166.52 cm and 175.02 cm, respectively. For observers at sea, visual staging based on gonadal appearance, while the simplest and quickest, is also the most subjective and uncertain. Accurate staging based on histology and other laboratory methods should be encouraged in the future.
**Key words:** billfish, size at 50% maturity, functional maturity data, physiological maturity data, Western Indian Ocean

## Introduction

In the Indian Ocean, the stock status of some billfish species is not optimistic (IOTC, 2023). Reliable stock assessments form the basis for developing sound conservation and management measures and maintaining population sustainability. $L_{50}$ (size at 50% maturity) is an important concept in fish reproductive biology and a critical parameter in integrated stock assessment models. Incorrect estimation of $L_{50}$ could potentially impact assessment outcomes, thereby introducing uncertainties in the management and recovery of billfish stocks.

The estimation of $L_{50}$ largely depends on the data sources and biological criteria used to determine maturity. Common methods include histology, measurements of oocyte size, staging based on the appearance of whole oocytes, staging based on the external appearance of the ovary, and gonad indices (West 1990). The accuracy of these methods in determining gonadal development stages can vary significantly among different fish species. Overall, while visual staging based on the external appearance of the ovary is the simplest and most rapid method, it is also the most subjective and therefore the least precise (West 1990). Unfortunately, for billfish species managed by the IOTC, there are currently only two working papers from CPCs that provide estimates of $L_{50}$ based on the gonadal appearance staging method used by observers on board. As expected, there are considerable differences among some species, such as the striped marlin (Girault et al. 2019; Zhou et al. 2019, Table 1).

These discrepancies may arise from how maturity stages are defined. Girault et al. (2019) explicitly stated they used the criteria from Sakun and Butskaya (1968) to classify stages of gonadal maturity. However, Zhou et al. (2019) only mentioned maturity stages being divided into six levels (I - undeveloped stage; II - early developing stage; III - later developing stage; IV - mature stage; V - spawned stage; VI - spent stage). Therefore, providing detailed information on specific gonadal maturity stages classified by gender is essential.

Typically, the estimation of $L_{50}$ would require collecting samples during the spawning period and using the presence of females with vitellogenic oocytes that are capable of spawning within the given year. This criterion for estimating $L_{50}$ is often referred to as functional maturity (Brown-Peterson et al., 2011). However, when spawning capable females are difficult to obtain due to restrictions to sampling effort or the species spawn year around, maturity can still be estimated based on physiological maturity, e.g., gonadotropin-dependent phase of ovarian development first indicated by cortical alveolar oocytes and found in the early developing stages (Wright, 2007). When the maturity stages are determined by examining the gonadal appearance, it can be difficult to distinguish whether the individual with early developing phase is a first-time spawner entering the reproductive cycle from the immature stage or repeat spawner that are already mature fish entering the reproductive period from the regenerating stage. We hypothesize that the estimated $L_{50}$ is sensitive to the treatment of these individual. Therefore, in this study, we re-examined the maturity raw data with emphasis on the stage-III fish and estimated $L_{50}$ for four billfish species based on functional and physiological maturity data, respectively.

The objectives of this working paper are: (1) to provide the specific criteria for gonadal maturity stages used by the Chinese Scientific Observer Program; (2) to analyze the lower jaw-fork length (LJFL) distribution ranges for each maturity stage; (3) to compare the estimated $L_{50}$ for four billfish species based on functional and physiological maturity data, respectively.

## Material and methods

### Observer data

The data used in this study were obtained from the Chinese Tuna Longline Fisheries Observer Program, specifically the fishery dataset for the western Indian Ocean. The Chinese tuna longline fleet consists of deep frozen tuna longline vessels and ice-fresh tuna longline vessels, and the target species are mainly bigeye tuna (*Thunnus obesus*) and albacore tuna (*Thunnus alalunga*), and the bycatch of billfishes includes blue marlin (*Makaira mazara*, BUM), striped marlin (*Tetrapturus audax*, MLS), the black marlin (*Istiompax indica*, BLM) and indo-pacific sailfish (*Istiophorus platypterus*, SFA) etc. The study period spanned from October 2013 to January 2019, and the main area is from 34°N–30°S, 40°E–83°E.

Observers recorded basic information such as the date, time, latitude, longitude, hook type, and bait type for each fishing trip and recorded individual biological information of the caught species, including lower jaw-fork length (LJFL), sex (male, female, unidentified), the stage of gonad development and at-haulback condition etc. According to the China Fisheries Observer

Training Manual, observers at sea classify both ovaries and testes into 6 stages based on the appearance of gonads. The specific staging standards are described in Table 2.

The estimation of was based on female blue marlin, striped marlin, black marlin, and indo-pacific sailfish with sample sizes of 160, 88, 33, and 55, respectively.

**Data analysis**

The selection of samples as immature and mature was based on the maturity stages. In previous studies, stages I-III were considered as immature, and stages IV-VI were considered as mature. We defined such a maturity as functional maturity. However, there is a possibility that some of the early-developing-stage individuals could be physiologically matured spawners which enter the reproductive period from the regenerating stage. Therefore, in this study, we adjusted the maturity status for the stage-III individuals whose length are larger than the 75% of the stage-III fish (i.e., largest 25% of the stage-III individuals) from immature to mature. By doing so, we assumed that larger stage-III fish are more likely to be physiologically matured. We define the adjusted maturity data as physiological maturity.

Maturity data for females, including the two types of maturity data (functional maturity and physiological maturity), were then summarized with a logistic regression, which was used to model the log odds of being mature as a function of length. Logistic regressions are fit using glm function in R ver. 4.3.0 with a binomial distribution and a logit-link function. The probability of a fish being mature (p) given the length is computed by Equation (1):

$$p = \frac{e^{\alpha+\beta x}}{1+e^{\alpha+\beta x}} \qquad (1)$$

Where $\alpha$ and $\beta$ are the two parameters of the logistic regression. Length-at-maturity (i.e., the length at which 50% of the fish are mature, $L_{50}$) can be computed by solving Equation 1 for x (p=0.5).

## Results and Discussion

**The size distribution frequency of the billfish samples**

In all samples of the four billfish species, regardless of sex, we found that the majority of gonads were classified as stage III-IV. The blue marlin exhibited the widest size range, with a clear progressive trend in size ranges corresponding to different stages of maturity (Figure 1). The other three billfish species, likely due to limited sample sizes, showed a noticeably smaller size range. Additionally, LJFLs of some samples that classified as stage III clearly exceeded those of stage IV and VI samples (Figure 1).

For males, the classification of testes appears to be more easy and distinct, with samples at different maturity stages showing a progressive pattern in size ranges. However, for Indo-Pacific sailfish and striped marlin, stage II is an exception, with some individuals appearing in the largest LJFL classes (Figure 2). In contrast, the size patterns for females are more ambiguous, with stage III ovaries of Indo-Pacific sailfish and striped marlin appearing in larger LJFL classes (Figure 3). Finally, the size distributions by sex are shown in Figure 4. For black marlin and blue marlin, tuna longline fisheries captured a significantly higher proportion of male individuals.

**$L_{50}$ estimated based on physiological and functional maturity data**

***Indo-pacific sailfish***

For physiological maturity data, we adjusted 8 stage-III individuals from immature to mature. The estimated $L_{50}$ based on physiological maturity data was 177.55 cm, which was lower than that estimated based functional maturity data (187.04 cm, Figure 5).

***Black marlin***

For physiological maturity data, we adjusted 1 stage-III individual from immature to mature. The estimated $L_{50}$ based on physiological maturity data was 172.67 cm, which was lower than that estimated based functional maturity data (174.59 cm, Figure 6).

***Striped marlin***

For physiological maturity data, we adjusted 6 stage-III individuals from immature to mature. The estimated $L_{50}$ based on physiological maturity data was 166.52 cm, which was lower than that estimated based functional maturity data (170.05 cm, Figure 7).

***Blue marlin***

For physiological maturity data, we adjusted 9 stage-III individuals from immature to mature. The estimated $L_{50}$ based on physiological maturity data was 175.02 cm, which was lower than that estimated based functional maturity data (179.95 cm, Figure 8).

**Acknowledgments**

We thank the National Data Centre for Distant-water Fisheries of China for providing the fishery observer data for this study. We are also deeply grateful for the collaboration of Mr. Wang Zhenhua, Mr. Liu Pan, Mr. Wang Jiaqi, and Mr. Geng Zhe in introducing the gonadal staging standards of Chinese scientific observers for billfish.

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**Table 1 Comparison of $L_{50}$ Estimates for Four Billfish Species in the Indian Ocean Region (Girault et al. 2019; Zhou et al. 2019)**

| Species | Sex | LJFL range of samples (cm) | | $L_{50}$ (cm) | |
|---|---|---|---|---|---|
| | | Girault et al. (2019) | Zhou et al. (2019) | Girault et al. | Zhou et al. |
| BLM | M | 141-280 | 162-272 | 185 | 179.1 |
| | F | 129-306 | | - | |
| BUM | M | 122-265 | 98-325 | - | 179.6 |
| | F | 168-282 | | - | 178.0 |
| MLS | M | 146-240 | 144-240 | - | 183.7 |
| | F | 147-277 | | 232 | 169.0 |
| SFA | M | 135-237 | 134-230 | 210 | 195.8 |
| | F | 137-270 | | 203 | |

Note: black marlin (*Istiompax indica*; BLM), blue marlin (*Makaira mazara*; BUM), striped marlin (*Tetrapturus audax*; MLS), indo-pacific sailfish (*Istiophorus platypterus*; SFA), lower jaw-fork length (LJFL)

**Table 2. Criteria for Gonadal Maturity Classification of Tuna and Billfish Species adopted by Chinese Observers (Chen et al. 2004)**

| Maturity stages | Male | Female |
|---|---|---|
| I | Gonads very small, thread-like in shape, and sex not visually distinguishable | Gonads very small, thread-like in shape, and sex not visually distinguishable |
| II | Testes extremely fine, flat and thread-like in appearance, but sex is distinguishable | The ovaries are elongated, slender in appearance, but sex is distinguishable |
| III | The testes are enlarged, with a slightly rounded cross-section, and no sperm inside | The ovaries are enlarged, but individual eggs cannot be seen with the naked eye inside the ovaries |
| IV | Pressing the testes easily causes sperm to be released | The ovaries continue to enlarge, and individual eggs can be observed with the naked eye |
| V | The testes are full, milky white in color, filled with sperm, and sperm freely flows out | The ovaries are swollen, filling the body cavity, with eggs appearing transparent or translucent, easily separable or loosely arranged inside the ovaries |
| VI | The testes are relaxed, congested or streaked with blood, with a dark red surface, and contain no sperm or only a small amount of sperm inside | The ovaries are atrophied, relaxed, and congested; their volume significantly reduced, with a small residual amount of eggs remaining inside |

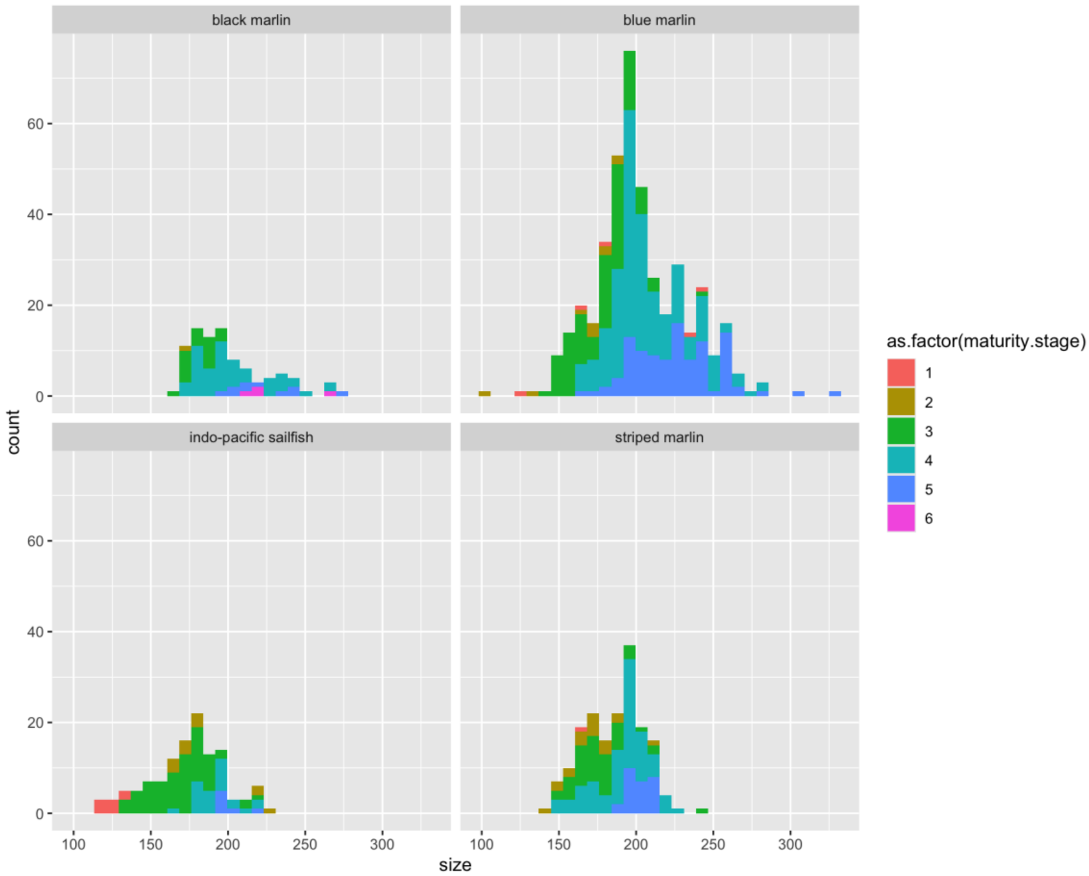



**Figure 1. Size frequency of maturity stages by species (male and female combined)**

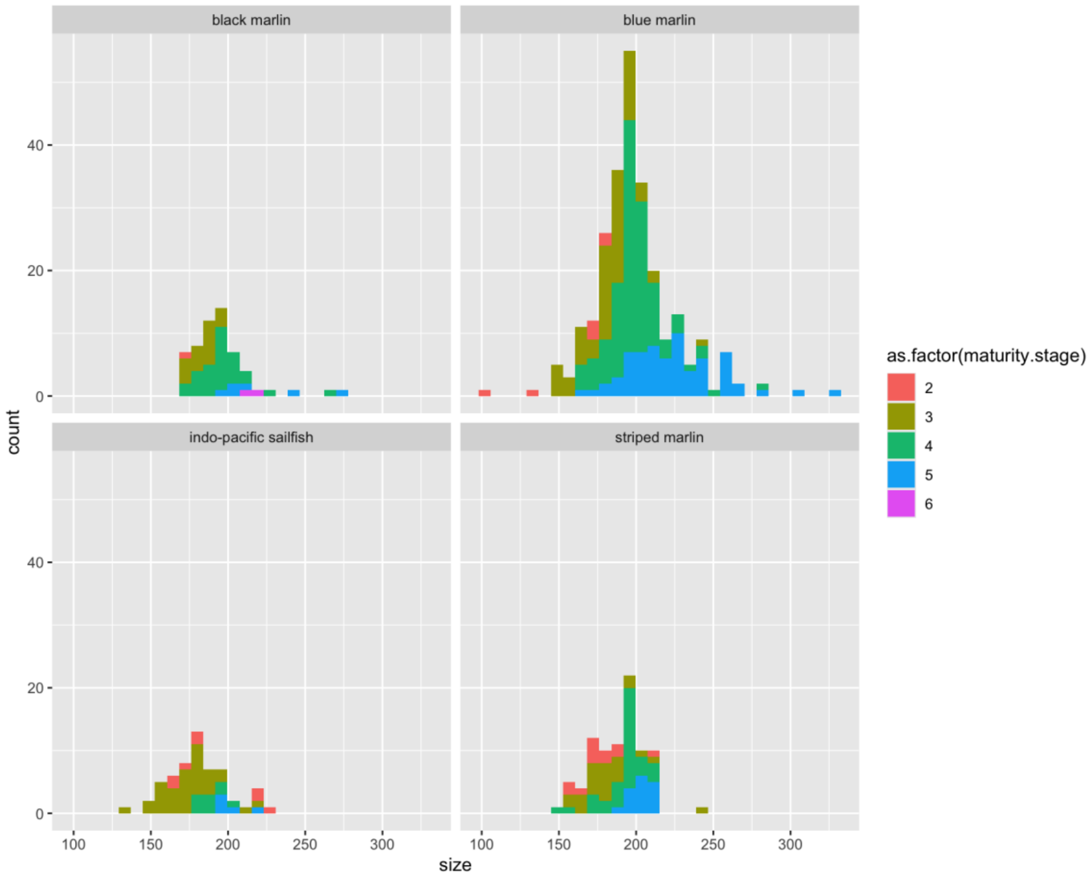



**Figure 2. Size frequency of male maturity stages**

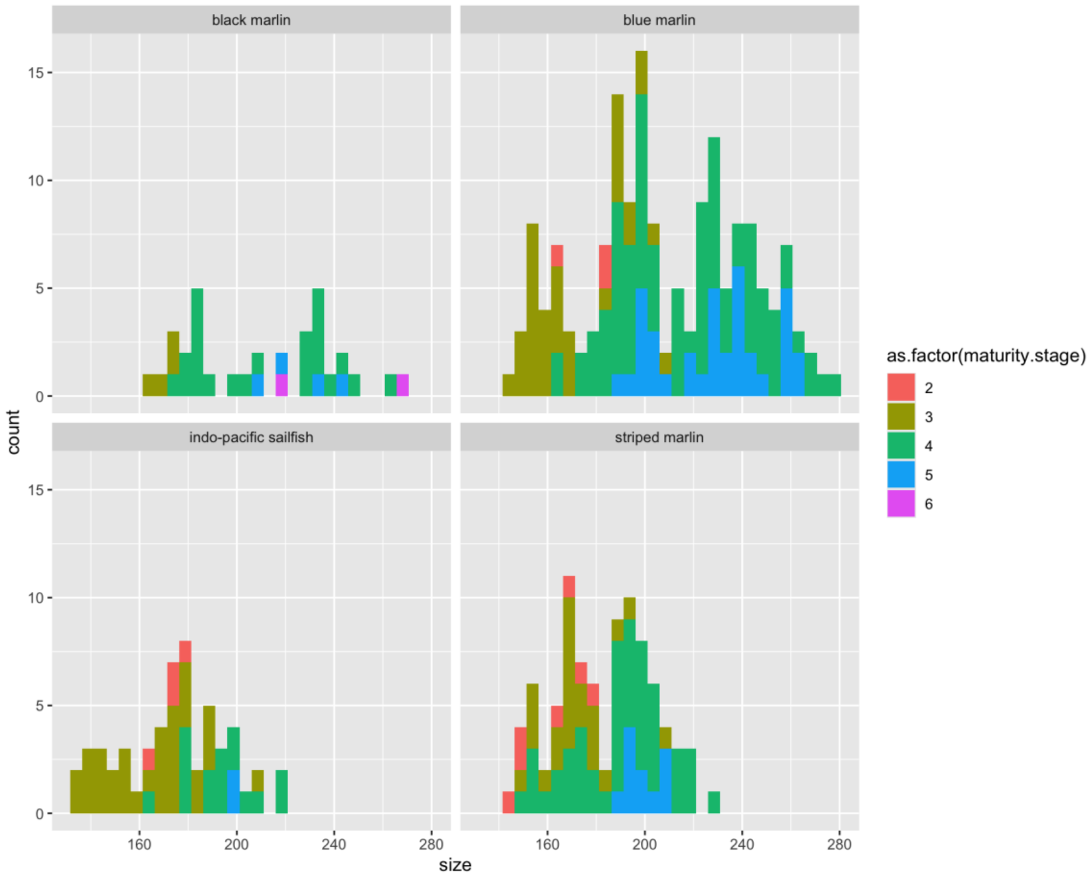



**Figure 3. Size frequency of female maturity stages**

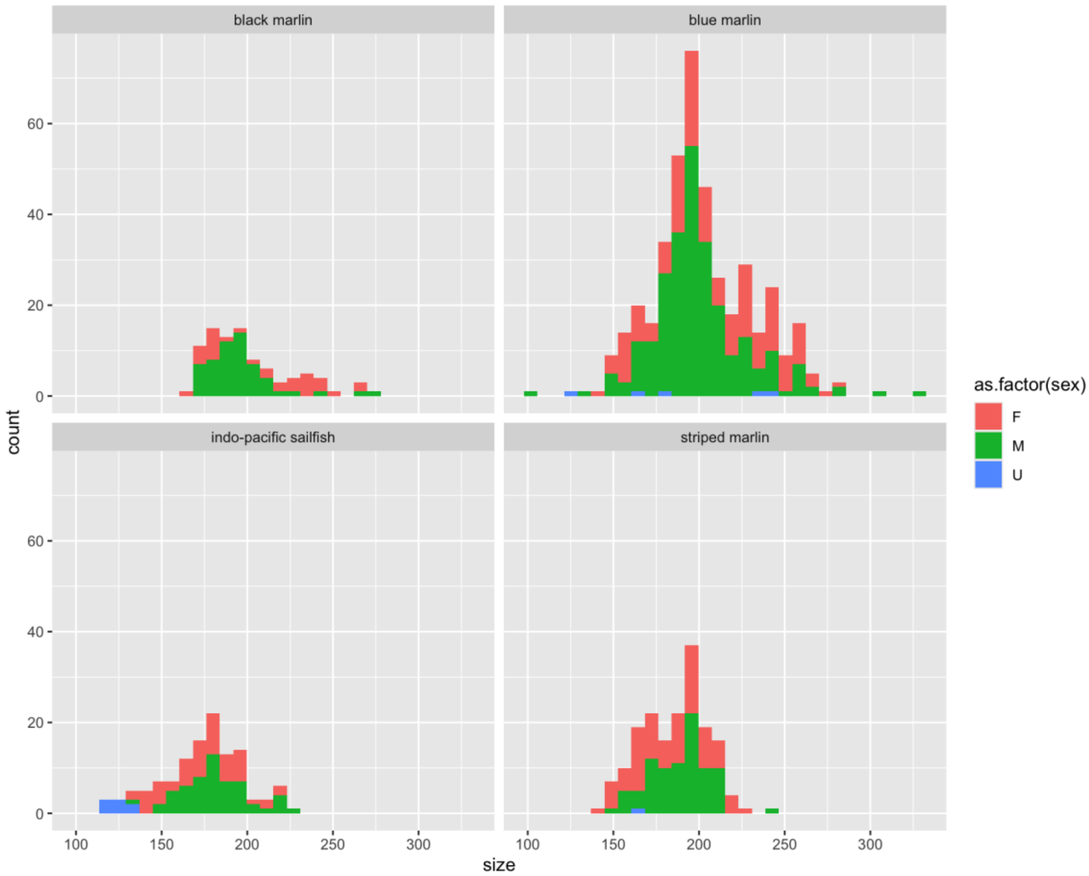
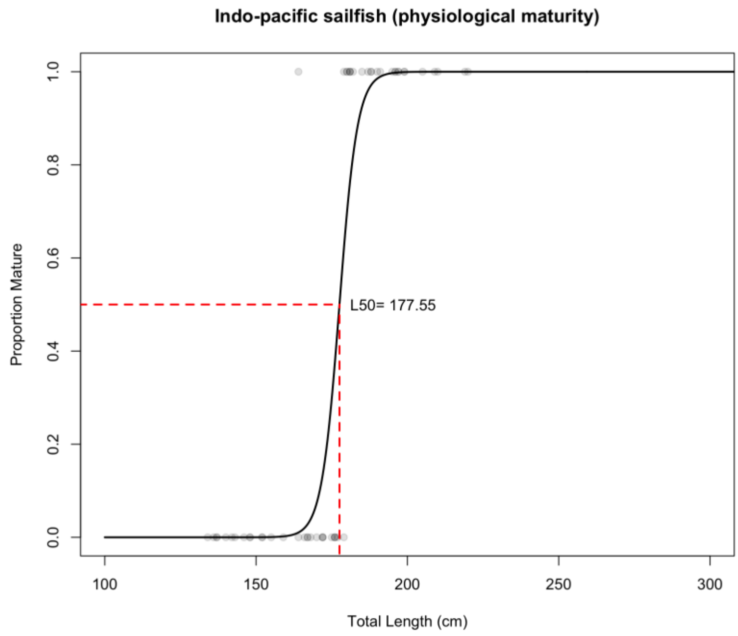



**Figure 4. Size frequency of sex by species**



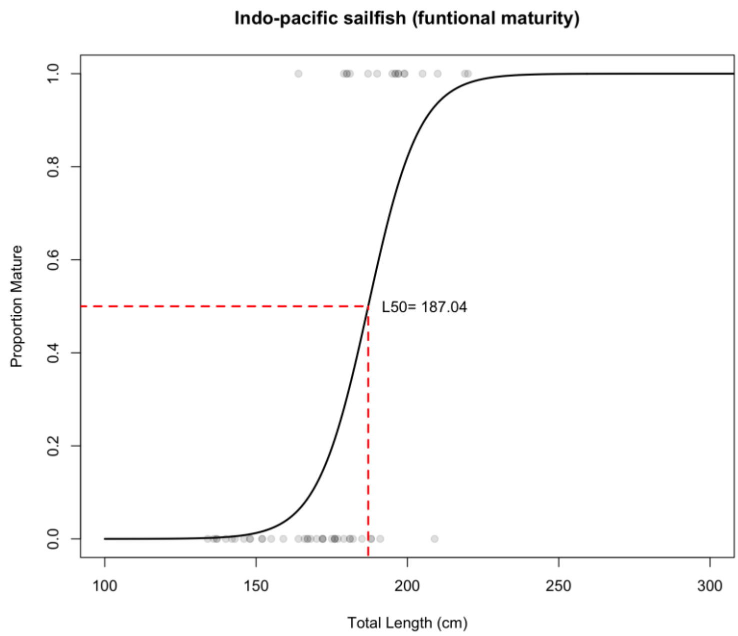




**Figure 5. Estimated length at which 50% of the fish are mature, $L_{50}$, based on physiological (upper) and functional (lower) maturity data for Indo-pacific sailfish.**

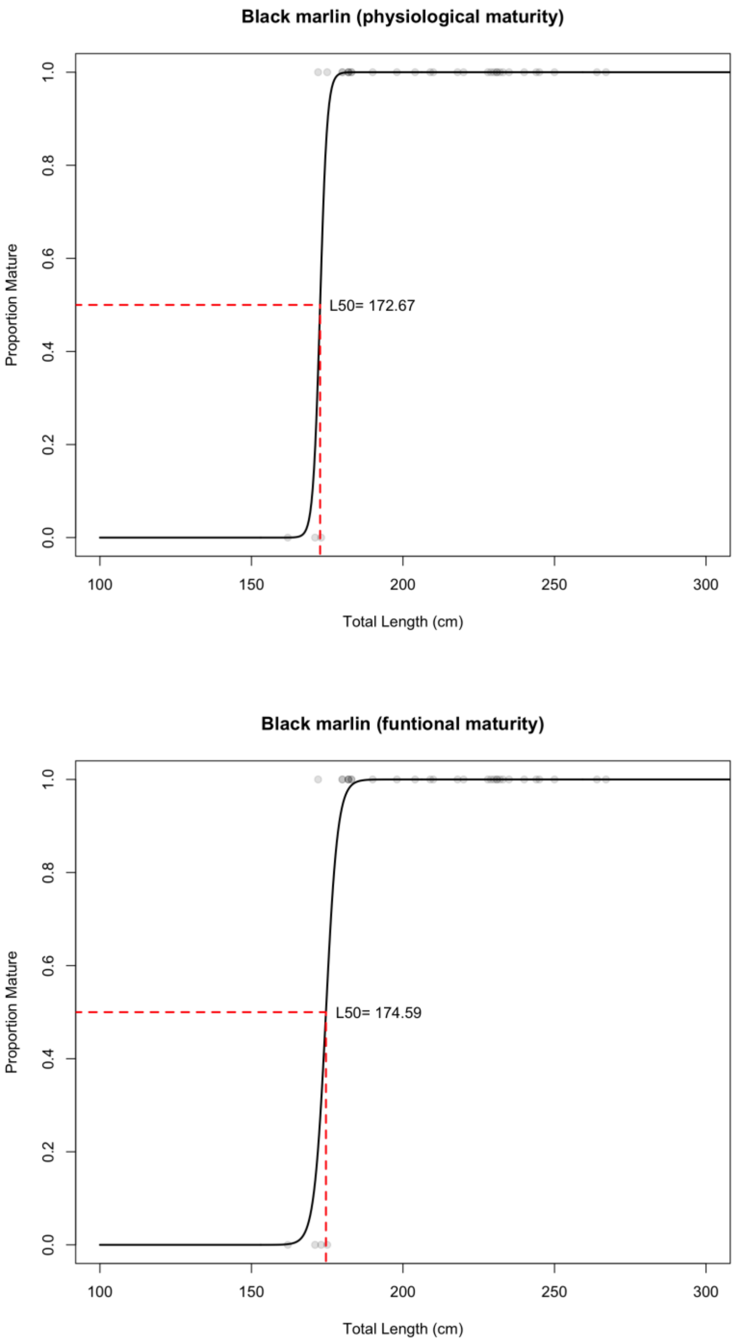



**Figure 6. Estimated length at which 50% of the fish are mature, $L_{50}$, based on physiological (upper) and functional (lower) maturity data for Black marlin.**

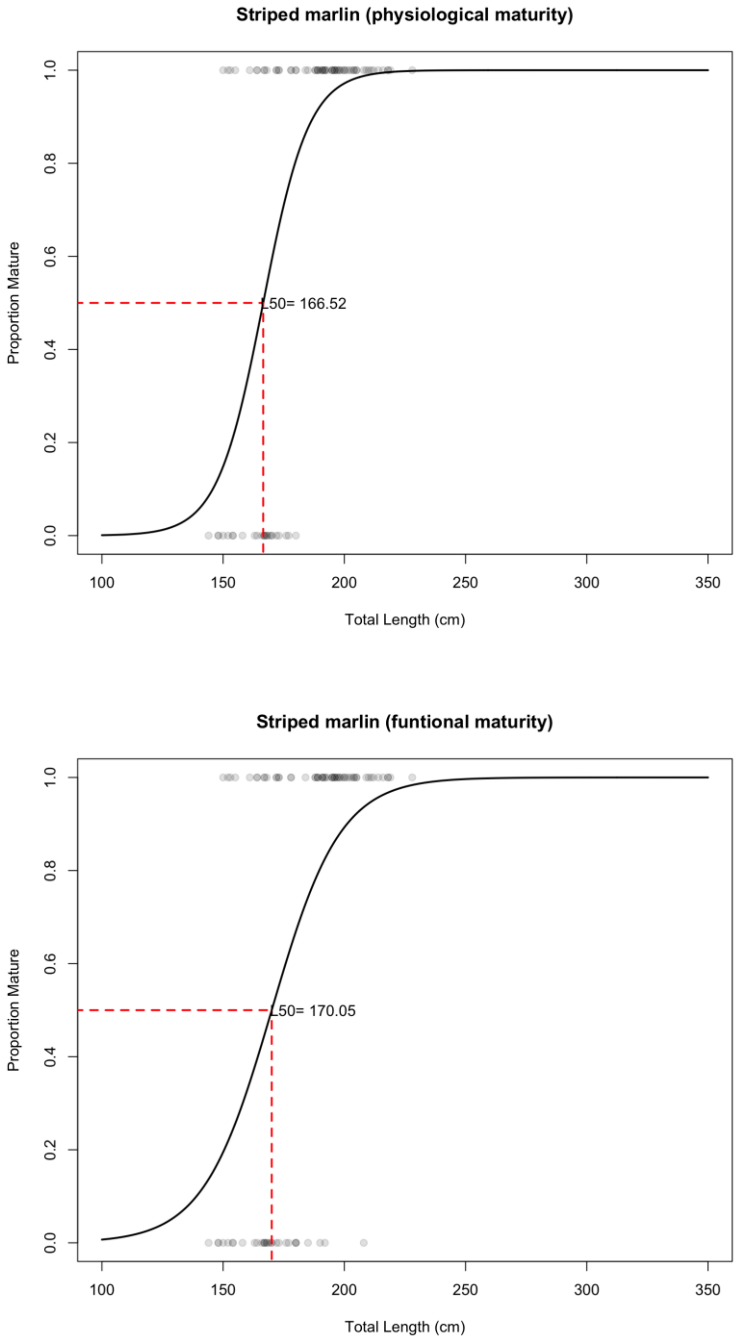
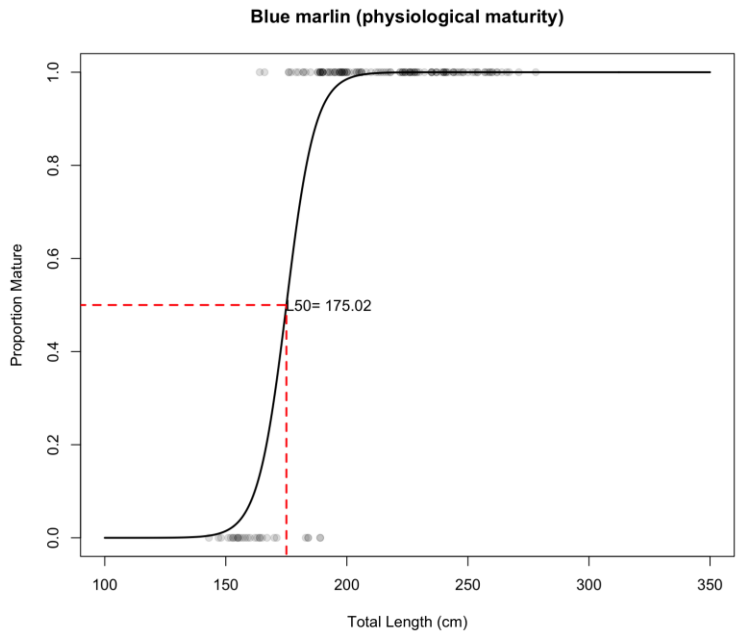



**Figure 7. Estimated length at which 50% of the fish are mature, $L_{50}$, based on physiological (upper) and functional (lower) maturity data for Striped marlin.**



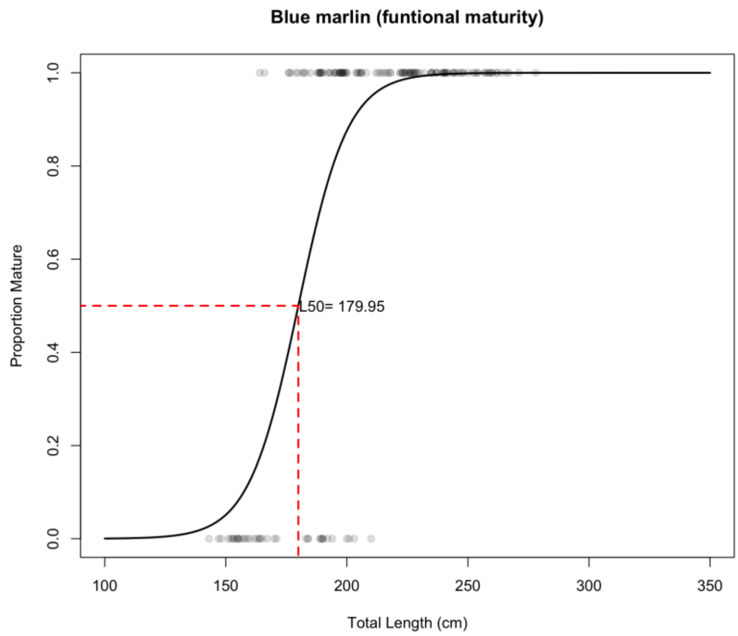




**Figure 8. Estimated length at which 50% of the fish are mature, $L_{50}$, based on physiological (upper) and functional (lower) maturity data for Blue marlin.**